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Carbon Isotope Discrimination, A Tool for Screening of Salinity Tolerance of Genotypes

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***Abstract*—**This study carried out in order to investigate the effects of salinity on carbon isotope discrimination (Δ) of shoots and roots of four sugar beet cultivars (cv) including Madison (British origin) and three Iranian culivars (7233-P_{12}, 7233-P_{21} and 7233-P_{29}). Plants were grown in sand culture medium in greenhouse conditions. Plants irrigated with saline water (tap water as control, 50 mM, 150 mM, 250 mM and 350 mM of NaCl + $CaCl_2$ in 5 to 1 molar ratio) from 4 leaves stage for 16 weeks. Carbon isotope discrimination significantly decreased with increasing salinity. Significant differences of Δ between shoot and root were observed in all cvs and all levels of salinity. Madison cv showed lower Δ in shoot and root than other three cvs at all levels of salinity expect control, but cv 7233-P_{29} had significantly higher Δ values at saline conditions of 150 mM and above. Therefore, Δ might be applicable, as a useful tool, for study of salinity tolerance of sugar beet genotypes.

***Keywords*—**Carbon isotope discrimination, Photosynthesis, Salt stress, Sugar beet

I. Introduction

SOIL salinity is a major abiotic stress affecting plant growth and productivity especially in arid and semi-arid areas such as Iran. Therefore, breeding for salinity tolerance can contribute significantly to crop yield in salt affected areas.

Most plants exposed to salinity show less CO_2 uptake by their leaves than the same plants not exposed to salinity. The photosynthetic capacity of plants grown under saline conditions is depressed depending on type of salinity, duration of treatment, species and plant age [1]. Many studies have concluded that the reduction in photosynthesis in response to salinity is to some extent the result of reduced stomatal conductance and consequently restriction of the availability of CO_2 for carboxylation [2},[3]. During the fixation of carbon by photosynthesis, the naturally occurring stable isotope ^{13}C is discriminated against, because of fractionation of carbon stable isotope (^{12}C and ^{13}C) mainly by Rubisco [4]. Plants therefore, contain a lower ratio of ^{13}C to ^{12}C than the air that supplies them [5].

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The most general model describing carbon isotope fractionation during photosynthesis in C_3 plants assumes that the major components contributing to the overall fractionation are the differential diffusion of CO_2 containing ^{12}C and ^{13}C across the stomatal pathway and the fractionation by Rubisco.

Farquhar et al. [6] reported that when stomatal conductance is small in relation to the capacity for CO_2 fixation, intercellular partial pressure of CO_2 (C_i) is also small and carbon isotope discrimination (Δ) tends toward 4.4‰ (α). When conductance is comparatively large, C_i approaches atmospheric CO_2 partial pressure (C_a) and Δ approaches net fractionation (b) between 27 to 30‰. Farquhar et al. [6] suggested the following expression for Δ in leaves of C_3 plants:

$$\Delta = \alpha + (b - \alpha)\, C_i/C_a \qquad (1)$$

Since the carbon incorporated in leaves is assimilated over a considerable time and under a range of environmental conditions, measuring Δ provides a long-term average estimate of C_i/C_a, and therefore is a long term indicator of plant metabolism.

Assessment of the possibility of using carbon isotope discrimination as an alternative method for selecting crop material might be time saving and beneficial. Therefore, the aims of present study were to investigate the effect of salinity on Δ of shoots and roots of four sugar beet cvs and evaluate the relationships between Δ, photosynthesis, dry matter and different plant parts.

II. Materials And Methods

Four sugar beet cvs differing in their salt sensitivity (Madison of British origin and Iranian cvs, 7233-P_{12}, 7233-P_{21} and 7233-P_{29} were grown in greenhouse conditions. Five levels of salinity (0, 50, 150, 250, and 350 mol m^{-3} of NaCl and $CaCl_2$ in 5:1 molar ratio) were imposed. Samples were taken after 16 weeks of salt treatment. Four plants of each cv in each level of salt treatment were harvested. Samples were placed in freezer at -87^oC and then freeze dried for 72h. The freeze dried samples were ground by mortar and pestle to pass a 2mm sieve. Ground samples of 1.0 to 1.5 mg were placed in a tin foil cap, taking care not to touch the foil during preparation. Carbon isotope composition ($\delta^{13}C$) of the shoot and root samples were analysed in a Europa Automated Nitrogen Carbon Analysis Solids/Liquids ANCA-SL System (Europe Ltd, Crewe.U.K). Carbon isotope discrimination was calculated as:

$$\boldsymbol{\Delta} = (\delta a - \delta p) / (1+\delta p) \qquad (2)$$

where δ*a* and δp are the carbon isotope composition of source air and plant material, respectively, relative to the international standard Pee Dee Belemnite (PDB) [7]. The values of δ*a* and δp were measured as described by Hubick et al. [7]. Isotope compositions are generally expressed as part per thousands, either ($x \times 10^{-3}$) or with the symbol (‰).

Intercellular and ambient CO_2 and net photosynthesis were measured by Combined Infra Red Gas Analysis System (CIRAS-1 portable photosynthesis system.

The data were subjected to balanced analysis of variance by Statistical Analysis System (SAS) Software for Windows version 6.12.

III. Results And Discussion

Carbon isotope discrimination (**Δ**) was analysed in shoots and roots of different sugar beet cvs. Isotope discrimination decreased with increasing salinity (Fig.1). The average shoot **Δ** of four cultivars was 22.803×10^{-3} and 19.82×10^{-3} at control and 350 mol m^{-3} salt, respectively. Madison cv showed lower **Δ** in shoot than other three cvs at all levels of salinity expect control, but P_{29} had significantly higher **Δ** values at saline conditions of 150 mol m^{-3} and above.

Carbon isotope discrimination in shoots was compared with the ratio of CO_2 partial pressure in intercellular air space and atmosphere (C_i/C_a), as obtained from gas exchange measurement during the lifespan of the plants. Fig. 2 shows relationships between **Δ** in shoots and C_i/C_a. A positive correlation was observed between C_i/C_a and **Δ** in shoots. Carbon isotope discrimination values of samples were much higher than the theoretical values calculated based on equation 1, but the slope of the observed line was less than that of the theoretical line (Fig.2). Plants in control conditions showed a higher C_i/C_a, which indicates higher stomatal conductance in these plants.

Analysis showed that roots in all cvs were isotopically heavier with respect to shoot. Thus, the discrimination was less in the roots than the shoots (Fig.1b). Madison and P_{21} cultivars significantly had lower **Δ** in control conditions, while P_{29} and P_{12} had highest **Δ** at the same condition. At saline conditions, cv P_{29} had significantly higher root **Δ** than others. At high salt concentration (350 mol m^{-3}) cultivars P_{29} and Madison had the highest and the lowest root **Δ**, respectively (Fig. 1b) and differences were significant. The best correlation, a linear regression line, was plotted between **Δ** in shoot and root. The results showed that at the all levels of salinity **Δ** in shoot is a good predictor for **Δ** in the root because the relationship between **Δ** in shoot and root is positively linear (r^2=0.79) (Fig. 3). Dry matter accumulation decreased with increasing salinity (data not shown) as **Δ** did. Therefore, there was a positive correlation between dry matter accumulation and **Δ** in the presence of salinity.

The best correlation, a parabolic regression line, was plotted between total dry weight (TDW) and **Δ** in shoot. The relationship between photosynthesis (A) and **Δ** is shown in Fig. 4a.

Shoot **Δ** increased with increasing photosynthesis in all cvs. However, there was a stronger correlation between photosynthesis and shoot **Δ** in cv Madison (r^2=0.95) than other cvs. Cultivar P_{29} showed a weaker correlation between photosynthesis and **Δ** (r^2=0.75). The relationship between **Δ** and stomatal conductance (g_s) is shown in Fig. 5. Shoot **Δ** increased with increasing g_s in all cultivars. However cv Madison in control condition showed a higher **A** and g_s as well as higher **Δ** than others.

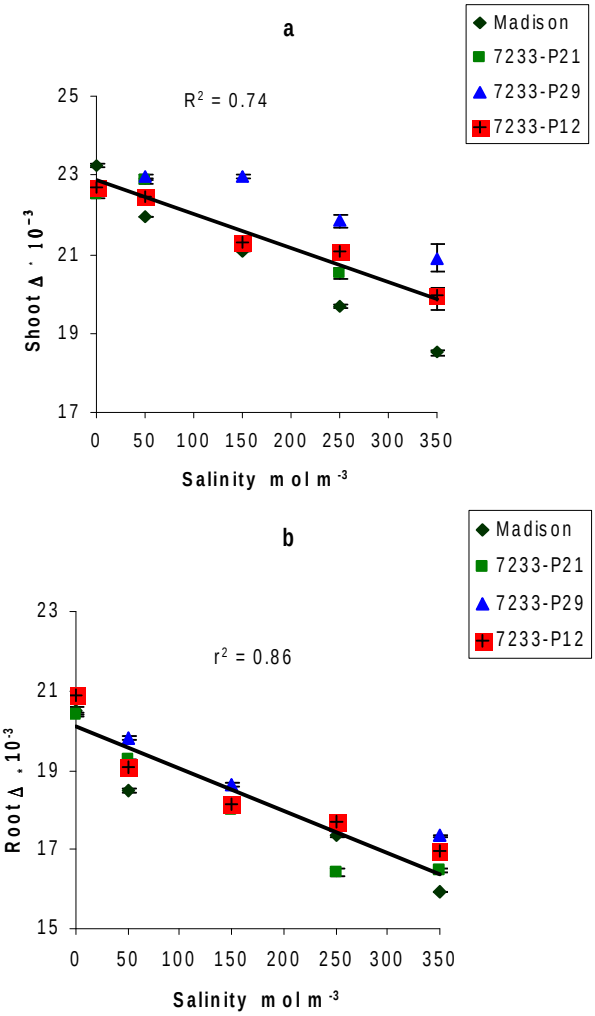

Fig. 1 Relationship between shoot (a) and root (b) carbon isotope discrimination (Δ× 10-3) and different levels of salt concentration. Each point is the average of four replications. Vertical lines are standard error of the means

The results of this investigation indicate that **Δ** could be used to select for salt tolerance in sugar beet cultivars. Although carbon isotope discrimination tended to be reduced by salinity, reduction in **Δ** was markedly greater at highest salinity (350 mol m^{-3}) than other salinity concentrations (Fig. 1).

The relationships of photosynthesis (**A**), **Δ** and g_s (Figs. 4 and 5) shows that one of the main causes of reduction in **A** at saline conditions is stomatal conductance which led to reduced discrimination [4], [8], [9].

The lower values of **Δ** in salt stressed plants in comparison with control suggest that reduction in CO_2 partial pressure of the intercellular space results from reduced stomatal conductance (g_s). This confirmed by the positive correlation (r^2=0.68) between stomatal conductance and **Δ** in the presence of salinity (Fig. 5). As expected from theory, a positive correlation was observed between shoot **Δ** and the average of C_i/C_a measured by gas exchange (Fig. 2). In shoots the slope of the line based on **Δ** measured in samples was less than that of a line drawn based on Equation 1 (Fig. 2). The scattering of data might be partly attributed to the measurements of C_i/C_a for a short period of photosynthesis, while the **Δ** values reflect carbon assimilation over a longer period [3]. In other words, although C_i/C_a spanned a wide range of values (from 0.2 to 0.8 across all treatments), measured organic **Δ** did not decrease to the values predicted from gas exchange. This is because gas exchange measurements were taken at one point in the experimental programme, but organic **Δ** integrates the history of the plant. Accordingly, if most structural carbon had been synthesised prior to any long-term effects of salinity then organic **Δ** values will be higher than those predicted. Additionally, because the absolute rate of assimilation was reduced to such an extent by the salinity treatment, only a small amount of carbon was available for export or new growth. The results of this experiment are in agreement with Brugnoli and Lauteri [8], who found a good correlation between **Δ** and C_i/C_a in cotton leaves (r^2=0.87). In this experiment the correlation between **Δ** and C_i/C_a was linear (r^2=0.72).

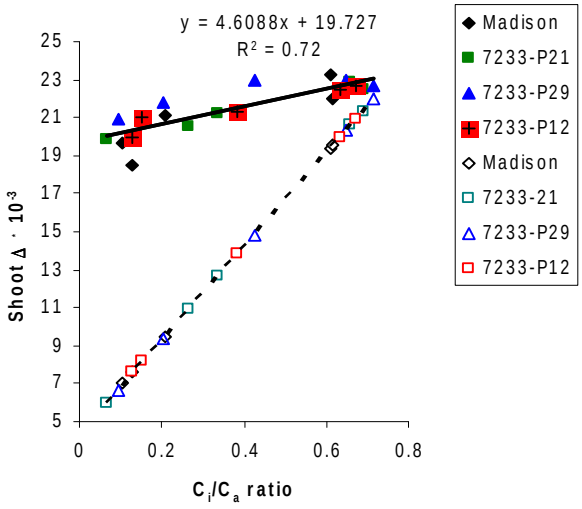

Fig. 2. Relationship between shoot carbon isotope discrimination ($\Delta\times10^{-3}$) and C_i/C_a ratio of sugar beet cvs in the presence of different levels of salinity. Closed symbol shows observed carbon discrimination and open symbol shows predicted carbon discrimination calculated from equation 1 with α=4.4‰ and b=29‰

Therefore, the indications are that stomatal conductance (g_s) had a greater effect than photosynthesis (**A**) on C_i. Cultivar P_{29} had significantly higher **Δ** than Madison at high salinity. Cultivar P_{29} did not show a higher g_s and C_i rather than Madison at saline conditions.

Therefore, the higher **Δ** in cv P_{29} under saline condition might be attributed to ability of this cv to re-use CO_2 produced by respiration because when stomata are closed due to water deficiency, there is less chance (almost no chance) for CO_2, produced by respiration, to escape to the atmosphere and the plant re-uses this CO_2. However, this type of CO_2, produced during respiration, is less enriched in ^{13}C because it has already passed through several physiological and biochemical processes that deplete its ^{13}C [2].

There was relatively more ^{13}C in roots than in shoots (Fig.1). Variations in **Δ** among different components within the plant have been reported by several authors in many species [3] ,[10] and have been attributed to different causes including fractionation during secondary metabolism. In general, secondary products are depleted in ^{13}C relative to primary compounds and catabolic reactions prefer the 'light' molecules, while the 'heavy' ones are involved in biomass formation.

Schmid and Gleixner [11] studied the isotopic correlations in wood as a system (cellulose and lignin). Cellulose as a polymer of primary products is ^{13}C-enriched relative to lignin, which is a secondary product. This confirmed the theory mentioned above. They also reported that secondary products in plants are depleted by 3-6‰ relative to primary compounds from the same source.

Accordingly, the ^{13}C-depletion in organic matter found in the present work could be caused by fractionation during non-photosynthetic processes. Indeed, the observed $\delta^{13}C$ in leaf material integrates different discrimination steps occurring during photosynthesis, photorespiration in the light, dark respiration and during export and translocation of photosynthetic products. Another possible reason for declining **Δ** in root material could be due to the time of producing carbohydrate for storage in the roots because with passing time **Δ** decreased in the presence of salinity [3]. Therefore, as most carbohydrates accumulated in the roots produced at the last stage of the plant's life, this might cause the reduction of **Δ** in the root.

In general, carbon isotope discrimination (**Δ**) decreased with increasing salinity and reduction in **Δ** values was related to decreases in **A**, E and g_s. The positive correlation between **Δ** and **A** or g_s is confirmed by previous reports in other crops like wheat [3]. Genotypic differences in **Δ** can be attributed to variation in photosynthesis and/or conductance of CO_2 to the sites of carboxylation [6] which is mainly controlled by stomata. In this experiment g_s had a main effect on **Δ**. Thus, carbon isotope analysis could be used in salinity studies of sugar beet cultivars. The salt-sensitive cv (Madison) showed lower **Δ** than salt tolerant ones (P_{29}). Therefore, carbon isotope discrimination might be a useful tool for study of salinity tolerance of different genotypes.

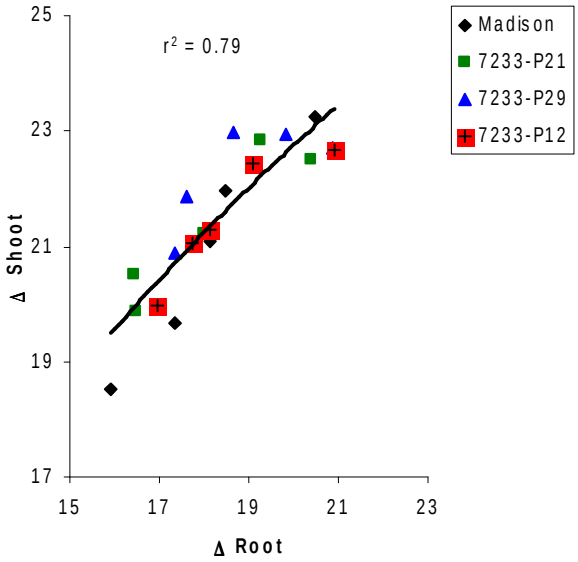

Fig. 3 Best fit relationship between shoot and root carbon isotope discrimination ($\Delta \times 10^{-3}$) of sugar beet cultivars in the presence of different levels of salinity. Each point is the average of four replications

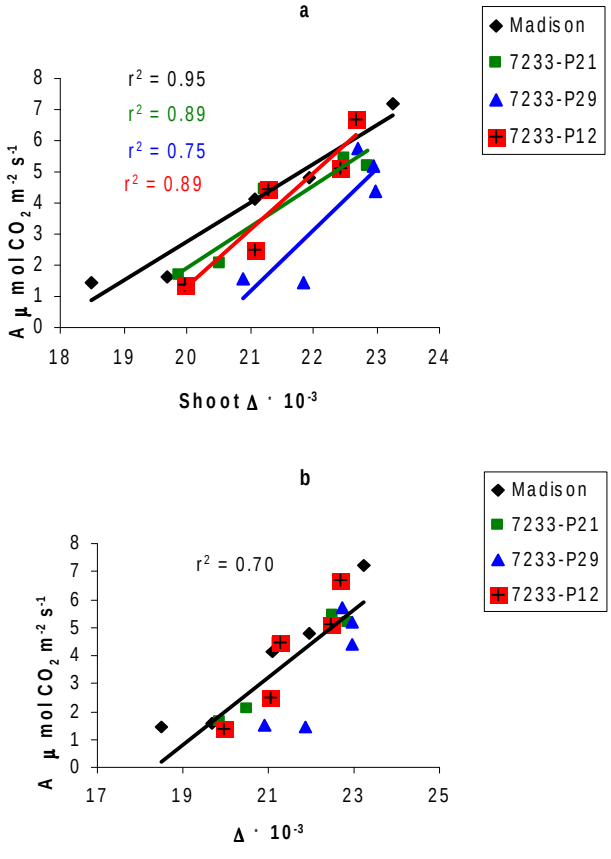

Fig. 4 Linear relationship between shoot carbon isotope discrimination (Δ) and photosynthesis (A). Top figure shows separate regressions for individual cultivars and the bottom figure shows overall regression for all cultivars

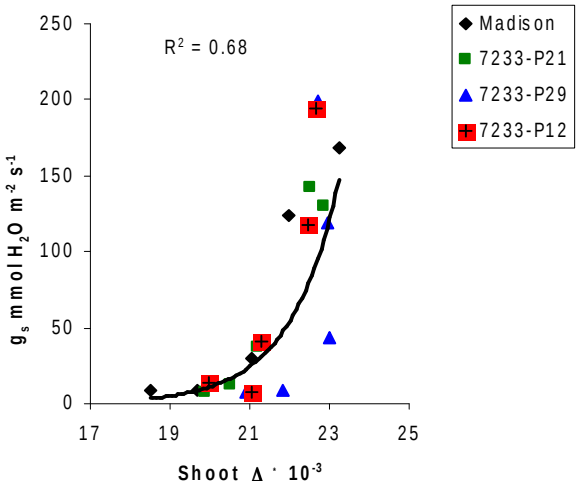

Fig. 5 Relationship between shoot carbon isotope discrimination (Δ) and stomatal conductance (g_s) of sugar beet cultivars in the presence of salinity. Each point is the average of four replications

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